AD_____

Award Number: DAMD17-00-1-0486

TITLE: Development of a Transgenic Mouse Model for Breast Cancer that is Optimized for the Study of T Cell-Based Therapeutic Strategies

PRINCIPAL INVESTIGATOR: Brad H. Nelson, Ph.D.

CONTRACTING ORGANIZATION: Virginia Mason Research Center
Seattle, Washington 98101-6525

REPORT DATE: June 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2002	Annual (15 May 01 - 14 May 02)

4. TITLE AND SUBTITLE
Development of a Transgenic Mouse Model for Breast Cancer that is Optimized for the Study of T Cell-Based Therapeutic Strategies

5. FUNDING NUMBERS
DAMD17-00-1-0486

6. AUTHOR(S)
Brad H. Nelson, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Virginia Mason Research Center
Seattle, Washington 98101-6525

E-Mail: bnelson@vmresearch.org

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Report contains color.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

Our goal is to develop a transgenic mouse model for breast cancer that will allow the *in vivo* activities of tumor-specific T cell clones to be tracked at all stages of tumorigenesis and after various immune interventions. We proposed to "tag" the *neu* oncogene with two defined T cell epitopes so as to confer recognition by available T cell receptor (TCR) transgenic T cells. When expressed as a transgene in mammary epithelium, epitope-tagged *neu* (designated $neu^{OT1/OT2}$) should induce formation of aggressive mammary adenocarcinomas that express the epitope tags and hence are recognizable by adoptively transferred TCR trangenic T cells. This past year, we have obtained five $neu^{OT1/OT2}$ transgene-positive founders, which have been bred to produce multiple female offspring. Expression of *neu* in mammary epithelium is being evaluated, and mice are being monitored for mammary tumorigenesis. Meanwhile, we have commenced adoptive T cell transfer experiments using a convenient, transplantable lymphoma model, and have discovered signaling differences between T cells that are responding to antigen-positive tumors versus the same antigen delivered with adjuvant. Thus, Aim 1 and much of Aim 2 have been successfully completed, and we are beginning to obtain novel insights into T cell responses in the tumor environment.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, transgenic mouse, tumor immunology, immunotherapy, CD4+ and CD8+ T lymphocytes, adoptive transfer, HER2/neu	8
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Cover 1

SF 298 2

Table of Contents 3

Introduction 4

Body 4

Key Research Accomplishments 5

Reportable Outcomes 6

Conclusions 6

References 6

Appendices 6

PI: Brad H. Nelson, Ph.D.

Title of Project: Development of a transgenic mouse model for breast cancer that is optimized for the study of T cell based therapeutic strategies

Introduction:

Currently, the development of immune-based therapies for breast cancer is impeded by the lack of an animal model that both mimics spontaneous human disease and is amenable to detailed monitoring of the activities of multiple, defined T cell clones that recognize tumor antigens. In this project, we are creating transgenic mice that are genetically programmed to develop spontaneous mammary tumors expressing defined T cell epitopes. Once such mice are available and tumors have developed, we will adoptively transfer CD4+ and CD8+ T cell clones with specificity for the engineered epitopes. These T cells will then be tracked in vivo and analyzed for functional responses to tumor cells. In future, this system will be used to evaluate the mechanisms and efficacy of immune-based therapeutic and preventative strategies. The specific aims of this proposal are:

(1) To construct a dual epitope-tagged version of the oncogene *neu* that is recognized by two epitope-specific T cell clones while retaining transforming potential;

(2) To assess the ability of epitope-tagged *neu*, upon expression in transgenic mice, to induce the formation of mammary tumors that are recognized by epitope-specific CD4+ and CD8+ T cell clones.

Body:

Aim 1: To construct a dual epitope-tagged version of the oncogene neu that is recognized by two epitope-specific T cell clones while retaining transforming potential.

Aim 1 was completed in its entirety during the first year of funding, as described in the 2001 Annual Report.

Aim 2: To assess the ability of epitope-tagged neu, upon expression in transgenic mice, to induce the formation of mammary tumors that are recognized by epitope-specific CD4+ and CD8+ T cell clones.

As proposed, the dual epitope-tagged version of *neu* was inserted into a transgenic expression vector containing the MMTV promoter (obtained from Dr. Tim Lane, UCLA). The insert and vector were thoroughly sequenced to ensure they contained no unintended mutations or alterations. Transgenic C57Bl/6 mice were generated by standard oocyte injection in the Dept. of Comparative Medicine at the University of Washington. Five transgene-positive founder pups were born (5 males, 2 females), and each has been bred to produce multiple offspring. Female offspring have been left to mature and are monitored regularly for mammary tumor formation, whereas male offspring are being culled shortly after birth (except those kept for breeding purposes). So far, none of the female founders or their offspring have developed palpable mammary tumors. The oldest transgene-positive females that have been monitored are now one year of age, whereas younger offspring from other founders are approximately 7 months of age. Thus, we can conclude at this point that the latency of tumor formation is likely greater than 7-12 months. We had hoped to identify a founder line that developed tumors between 100-300 days of age, which is still a possibility for some founders. However, we are also beginning to implement the following alternative strategies to try to decrease the latency of tumor formation:

(1) Some founders are being bred to mice of the FVB strain, as FVB mice are highly susceptible to *neu*-induced tumorigenesis. We expect C57Bl/6 x FVB F1 mice will have a shorter latency of tumor formation than pure C57Bl/6 mice.

(2) Founders will soon be bred to FVB mice that express a dominant-negative p53 transgene in mammary epithelium (under the control of the whey acidic protein promoter). This dominant-negative form of p53 has been shown by other groups to greatly accelerate *neu*-induced tumorigenesis. It has taken several months to obtain a license from Dupont to use these mice, but that has now been accomplished and we expect to set up these breedings in the next few months.

In the next month, we are also going to sacrifice representative female mice from each founder line and assess expression of the epitope-tagged *neu* transgene in mammary epithelium by Northern blot or RNAse protection assay. This will help us decide which founder lines to focus on for the breedings described above.

While we wait for our transgenic mice to develop tumors, we have forged ahead with adoptive T cell transfer experiments, similar to what was described in Aim 2 of the original proposal. Our goals are two-fold: (1) to hone our skills at adoptive T cell transfers and flow cytometry, which are technically demanding, and (2) to further refine the experimental questions that will eventually be addressed in the mammary tumor model. To this end, we have used a well-characterized lymphoma model involving the EL-4 cell line transfected to express the model antigen chicken ovalbumin (OVA). Mice bearing small EL-4/OVA tumors have been infused with CD8+ OT-I TCR transgenic T cells (which recognize OVA, as described in the original proposal). The T cells have been pre-labeled with the vital flourescent dye CFSE so that they can later be recovered and analyzed by flow cytometry. So far, we have learned that the OT-I T cells undergo a vigorous proliferative response to the EL-4/OVA tumor (Figure 1). Unexpectedly, this occurs without expression of the the canonical T cell activation marker CD69, implying that the activation status of these T cells is abnormal (Figure 2). Furthermore, these proliferating T cells fail to express the interleukin-2 (IL-2) receptor alpha chain (CD25), which implies that this is an IL-2-independent proliferative response (Figure 2). By contrast, when non-tumor-bearing mice are vaccinated with OVA protein in complete Freund's adjuvant (which delivers a strong, conventional immune stimulus), the OT-I T cells proliferate equally well but also become positive for CD25 (data not shown). Therefore, we are currently investigating the hypothesis that tumor-derived antigen may trigger an abnormal activation program in T cells that is associated with robust proliferation in the absence of IL-2 signaling, whereas conventional antigen delivery induces proliferation that is associated with IL-2 signaling. If this proves to be the case, we will next evaluate the effect of IL-2 signaling, versus the lack thereof, on the lifespan and functional activity of the CD8+ T cells. Ultimately, we intend to also address these issues in the mammary tumor model.

Key Research Accomplishments:

The following items have been completed or are underway:

Task 1. To construct a dual epitope-tagged version of the oncogene *neu* that is recognized by two epitope-specific T cell clones while retaining transforming potential (Months 1-12). ***completed**

a. Construct plasmids encoding single ($neu^{IE\square}$ and neu^{OVA}) and dual ($neu^{IE\square/OVA}$) epitope-tagged versions of *neu*; verify DNA sequence (Months 1-3). ***completed**
b. Evaluate signaling and transforming properties of epitope-tagged and untagged versions of *neu* in cell lines; if problems noted, modify epitopes as needed (Months 4-12). ***completed**
c. In vitro assays to evaluate recognition of IE□ and OVA epitopes by CD4+ and CD8+ T cells from TCR-transgenic mice (Months 4-12). ***completed**

Task 2. To assess the ability of epitope-tagged *neu*, upon expression in transgenic mice, to induce the formation of mammary tumors that are recognized by epitope-specific CD4+ and CD8+ T cell clones (Months 13-36). ***underway**

a. Construct MMTV vector with human growth hormone gene at 3' end into which to introduce *neu* transgenes (Months 9-12). ***completed***
b. Insert untagged (*neu*) and dual tagged ($neu^{IE\Box/OVA}$) cDNAs into MMTV vector (Month 13). ***completed***
c. Provide transgenes to the Dept. of Immunology at the University of Washington and have C57Bl/6 transgenic founder mice generated (Months 14-17). ***completed***
d. Perform PCR on tail DNA of pups (approximately 60 animals); breed transgene-positive founders (10-12 animals) (Months 18-19). ***completed***
e. Expect birth of F2 generation (50-100 animals); perform PCR on tail DNA; cull males (Month 20). ***completed***
f. Monitor female F2 mice for tumor formation (25-50 animals); as tumors develop, perform autopsies and tumor histology with the Dept. of Comparative Medicine at the Univ. of Washington (Months 22-32). ***underway***
g. Perform adoptive T cell transfer experiments on animals that develop tumors (approximately 35 *neu* mice, 35 $neu^{IE\alpha/OVA}$ mice, 20 IEα-specific TCR transgenic mice, and 20 OVA-specific TCR transgenic mice (Months 30-36). ***underway using a lymphoma model***

Reportable Outcomes:

Poster:
The elements that augment and limit tumor-specific CD8+ lymphocyte responses in vivo. Richard M. Tempero, Marc D. Coltrera, and Brad H. Nelson. Abstract #5497, 93rd Annual Meeting of the American Association for Cancer Research, San Francisco CA, April 6-10, 2002.

Invited presentation:
Molecular control of T cell proliferation in response to tumors. Brad H. Nelson. Annual Meeting of the British Columbia Cancer Agency, Vancouver, BC, Canada, November 23-24, 2001.

Conclusions:

The mouse model we are developing should lead to an improved understanding of the immune response to breast cancer and may facilitate the development of novel immune-based therapies or immunopreventive strategies for this disease. Toward this goal, we have now created a dually epitope-tagged version of *neu* that is recognized by the appropriate CD4+ and CD8+ T cells while retaining transforming potential. The generation of transgenic mice expressing this version of *neu* in mammary epithelium is underway, with five transgene-positive founder lines obtained to date. Adoptive T cell studies have been started using a convenient lymphoma model, and our preliminary results suggest the intriguing possibility that tumors may trigger a different T cell activation program than conventional antigenic stimuli. In Year 3, we will continue to pursue the goals outlined in Aim 2 of our original proposal, and will implement two alternative strategies to try to decrease the latency of tumor formation (as described above). No other changes to the research plan are expected. In summary, this study is on schedule and no major obstacles have been encountered.

References:

None.

Appendices:

See accompanying Figures 1 and 2.

Figure 1. OVA-specific CD8+ T cells proliferate upon recognition of an EL-4 lymphoma expressing OVA antigen. Mice bearing a palpable, subcutaneous OVA-negative (left panels) or OVA-positive (right panels) EL4 lymphoma were infused by tail vein injection with 5 x 10^6 naive OT-I TCR transgenic T cells that were pre-labeled with the flourescent dye CFSE. Three days later, lymphocytes were isloated from lymph nodes draining the tumor bed, stained with PE-conjugated anti-CD8, and analyzed by flow cytometry. Proliferating T cells can be identified by their diminishing CFSE intensity with each cell division (circled populations of cells). As shown here, OT-I cells proliferate vigorously in response to OVA-positive tumors, but not OVA-negative tumors, demonstrating that this is antigen-induced proliferation.

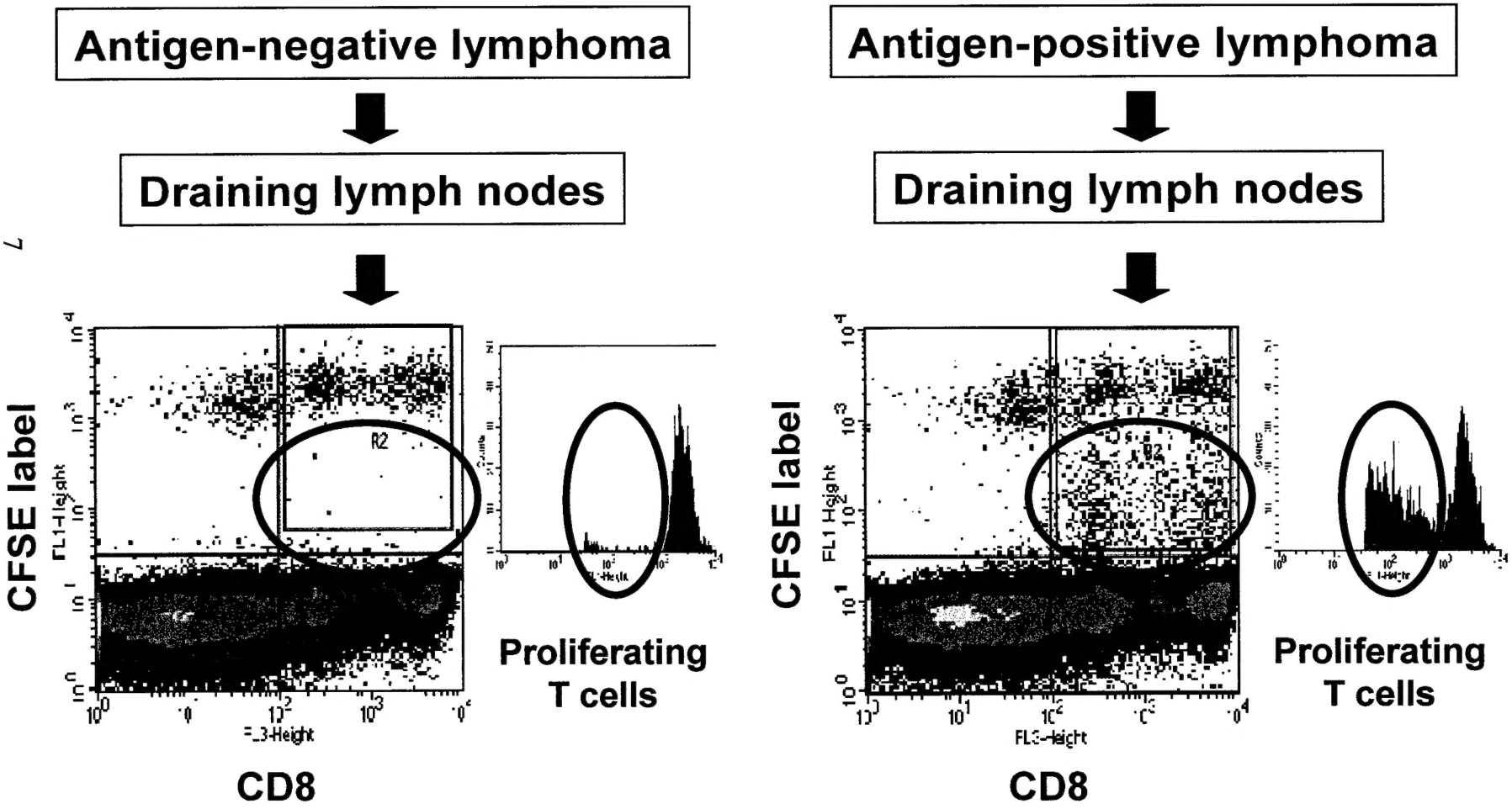

Figure 2. OVA-specific CD8+ T cells show incomplete activation upon tumor recognition. Lymphocytes prepared as in Figure 1, from a mouse bearing an OVA-positive tumor, were further analyzed by flow cytometry for cell size and expression of multiple activation markers. The upper row of panels shows results for the non-proliferating subset, and the lower row refers to the proliferating subset. The results show that proliferating OT-I T cells demonstrate several 'normal' features of activated T cells, including increased cell size, upregulation of CD44, and downregulation of CD62L. However, these cells fail to upregulate IL-2Ralpha or CD69 expression, leading to the conclusion that they are abnormally or incompletely activated.